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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 17/08, A61K 31/70 — A1

(11) International Publication Number: WO 96/19489

(43) International Publication Date: 27 June 1996 (27.06.96)

(21) International Application Number: PCT/IE95/00065

(22) International Filing Date: 19 December 1995 (19.12.95)

(30) Priority Data:
940973 19 December 1994 (19.12.94) IE

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(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DE (Utility model), DK, DK (Utility model), EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMPOUNDS

(57) Abstract

Antibiotic and mucolytic salts of roxithromycin, clarithromycin and azithromycin are described. Particularly described is roxithromycin stinoprate which was found to be more potent than erythromycin stinoprate.

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"Compounds"

Introduction

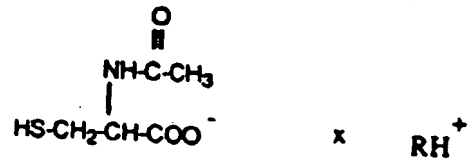
The present invention relates to new antibiotic and mucolytic salts of roxithromycin, clarithromycin and azithromcyin.

The exploitation of the therapeutical properties of thiolic compounds in combination with the properties of antibiotics has been attempted as described in EP 0,057,489A. However, it has been found that acetyl-cysteine and the derivatives thereof are relatively unstable and especially sensitive to oxygen, sunlight, humidity and heat.

The invention is directed towards providing antibiotic and mucolytic salts.

Statements of Invention

The derivatives according to the present invention have the following general formula :



wherein R is a radical selected from :

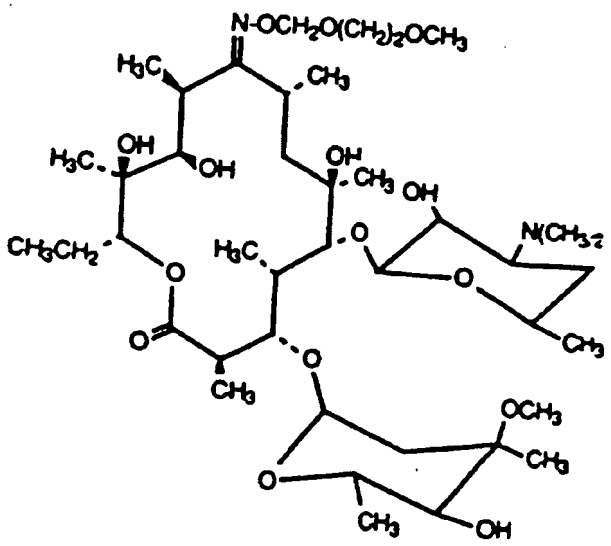
Roxithromycin, Clarithromycin and Azithromycin.

It has been surprisingly found that the compounds of the present invention are very stable, have very low toxicity and can be therapeutically used once daily.

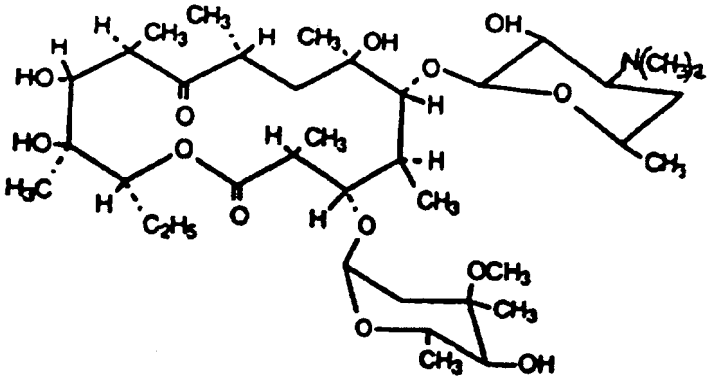
These new derivatives of the below mentioned macrolides have the further advantages of :-

(a) better oral absorption;
(b) faster and superior concentration;
(c) slow elimination; and/or
(d) reduced dosage : only 300 - 500 mg per day.

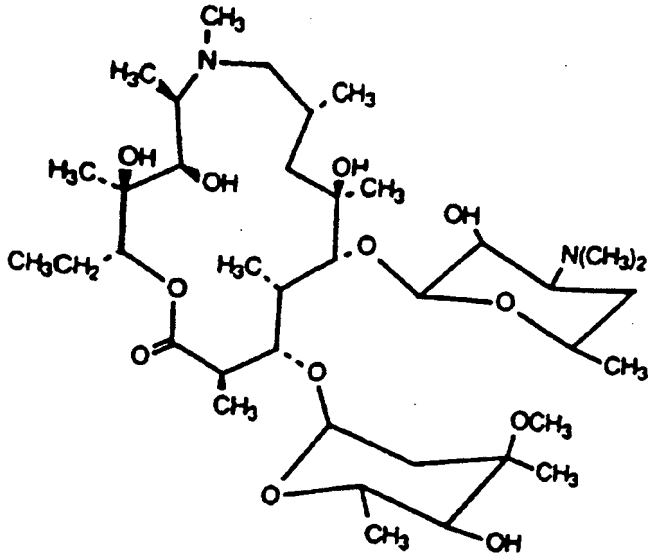
Roxithromycin has the structure



Clarithromycin has the structure



Azithromycin has the structure



The compounds according to the invention are white microcrystalline powders.

Their use is foreseen in all pharmaceutical forms and the compounds may be provided in any suitable pharmaceutical composition including : capsules; solutions; injectable preparations; aerosols; effervescent tablets; powders; creams; and suspensions. The pharmaceutical compositions will typically contain suitable excipients and/or vehicles which are conventionally used in galenical pharmacy.

The method for the preparation of the new salts comprises reacting roxithromycin, clarithromycin or azithromycin base with N-acetylcystein in a stoichiometrical ratio or preferably with a slight excess of the antibiotic nucleus. Most preferably the reaction is carried out in an organic solvent, at a temperature of between 20 and 40°C and in the presence of water, preferably in an amount of not greater than 20%. Alternatively, the reactions may be carried out in a suspension of water at a temperature of 20 and 40°C, (with N-acetylcystein in a stoichiometrical ratio or in

the presence of a slight excess of the antibiotic nucleus), and after building the salts, water is distilled off under very mild conditions (low vacuum, low temperature).

Example

Salt of roxithromycin with N-acetylcystein

1800 g of Roxithromycin and 350.9 g of N-acetylcystein are homogenised under inert conditions (nitrogen) for 60 minutes at 15-20°C. To this mixture 720 ml of deionised water is added at atmospheric pressure and further homogenised for one hour at 15-20°C. The product is dried under vacuum and milled, if necessary. A yield of 79.8% (1716 g) is obtained.

The Infra Red spectrum of the compound is plotted in Fig. 1.

The salts of clarithromycin and azithromycin are produced in a similar manner to that described in the above example.

MICROBIOLOGICAL ASSAY OF ROXITHROMYCIN STINOPRATE

References: USP 23 <81> Antibiotics-Microbial Assays, page 1690-1696 Code of Federal Regulations Title 21 :436.100 - 436.106

Test Organism:

Staphylococcus aureus ATCC 29737

The test organism was maintained through periodic inoculations on agar slants containing USP 23 Medium No. 1. The slants are incubated at 32-35°C for 24 hours, and

stored under refrigeration.

Assay Receptacles:

Sterile plastic petri-dishes (ca. 20 x 100mm) with covers were used as assay plates. Assay cylinders were manufactured from stainless steel (o.d. 8mm ± 0.1mm, i.d. 6mm ± 0.1mm, length 10mm ± 0.1mm).

Inoculum Preparation:

Using 5ml of sterile USP Saline T.S. the growth from an agar slant of S.aureus was washed and made up to 50ml with sterile saline solution. This stock suspension was diluted with sterile saline so that the transmittance, at 580nm, was 25% against saline as the blank.

1ml of this solution (i.e. giving 25% transmission) was added to each 100ml of culture media (USP 23 Medium No. 1 (Oxoid Antibiotic Medium No. 1)).

Culture Medium:

Oxoid Antibiotic Medium No. 1.

Solutions: Saline Solution (sterile)

Sodium chloride	0.9g
Purified Water	100ml

Sterilised at 121°C for 20 minutes.

Buffer Solution No. 3 (USP 23)

(0.1M potassium phosphate buffer pH 8.0)

Dibasic potassium phosphate	16.73g
Monobasic potassium phosphate	0.523g

Purified Water 1000ml

Adjusted with 10N potassium hydroxide to give a pH 7.9-8.1. Sterilised at 121°C for 20 minutes.

Standards: Erythromycin Stinoprate Standards:

About 100mg of Erythromycin Stinoprate standard, accurately weighed, was added to a 100ml volumetric flask. 10ml of methanol was added to dissolve the Erythromycin Stinoprate. This solution contains 10mg/ml of Erythromycin Stinoprate. A 1:10 dilution of this solution was then prepared with sterile buffer solution to obtain a 1000 µg/ml Erythromycin Stinoprate solution.

From this stock solution the following dilutions were prepared:
1.56 µg/ml (S_5); 1.25 µg/ml (S_4); 1.0 µg/ml (S_3); 0.8 µg/ml (S_2); and 0.64 µg/ml (S_1) (using sterile buffer solution for dilutions)

Test Sample (Roxithromycin Stinoprate):

About 100mg of Roxithromycin embonate test substance, accurately weighed, was added to a 100ml volumetric flask. 10ml of methanol was added for dissolution and this in turn was diluted with sterile buffer solution to obtain a 1000 µg/ml Roxithromycin Stinoprate solution.

From this stock solution a solution was prepared containing 1.0 µg/ml Roxithromycin Stinoprate, using sterile buffer solution for dilution purposes (U_3).

Method: Cylinder-Plate Method USP 23 <81>
Approximately 20ml of sterilised Oxoid Antibiotic Medium No. 1 was placed in each of 22 sterile petri-dishes, and allowed to harden. Using the inoculum described above 5.0ml of seed layer inoculum were added to each plate except two plates which were reserved as "negative controls". Six (6) stainless-steel assay cylinders were dropped on the inoculated surfaces of 18 of the plates from a height of 12mm, with even spacing on a radius of 28mm. The remaining two inoculated plates were retained as positive controls.

Using a 1-level assay with standard curve, alternate cylinders on each of three plates were filled with the 1.0 µg/ml solution of Erythromycin Stinoprate (S_3), and each of the remaining nine cylinders were filled with one of the four other dilutions of the Standard (S_1 - S_5). This process was repeated for the other three dilutions of the standard.

The 1.0 µg/ml solution of Erythromycin Stinoprate (S_3) was filled into alternate cylinders on each of three plates and

the remaining nine cylinders were filled with the Test Sample (U_3).

Incubation: 24 hours at 32-35°C

Estimation of Potency:

The potency of the Roxithromycin Stinoprate test substance was compared to the standard curve obtained for the Erythromycin Stinoprate standard dilutions. This was calculated as a percentage of the potency of the 1.0 µg/ml Erythromycin Stinoprate standard dilution.

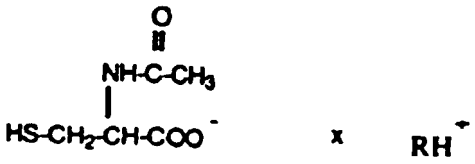
Results: Roxithromycin Stinoprate

Mean potency ($\bar{x}$) = 114% (of Erythromycin Stinoprate).
Standard deviation (s.d.) = 2.94%.
Coefficient of variation (c.v.) = 2.56%.

The invention is not limited to the embodiments hereinbefore described which may be varied in detail.

CLAIMS

1. A compound of the formula



wherein R is a radical selected from :

Roxithromycin, Clarithromycin and Azithromycin.

2. N-Acetylcystein-Roxithromycin Salt.

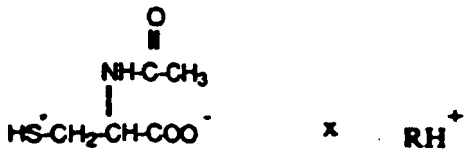
3. N-Acetylcystein-Clarithromycin Salt.

4. N-Acetylcystein-Azithromycin Salt.

5. A compound substantially as hereinbefore described with reference to the examples.

6. A pharmaceutical composition comprising a compound as claimed in any preceding claim together with at least one pharmaceutically acceptable excipient and/or carrier.

7. A process for preparing a compound of the formula



wherein R is a radical selected from :

Roxithromycin, Clarithromycin and Azithromycin.

comprising the step of reacting N-Acetylcystein with roxithromycin, clarithromycin or azithromycin base.

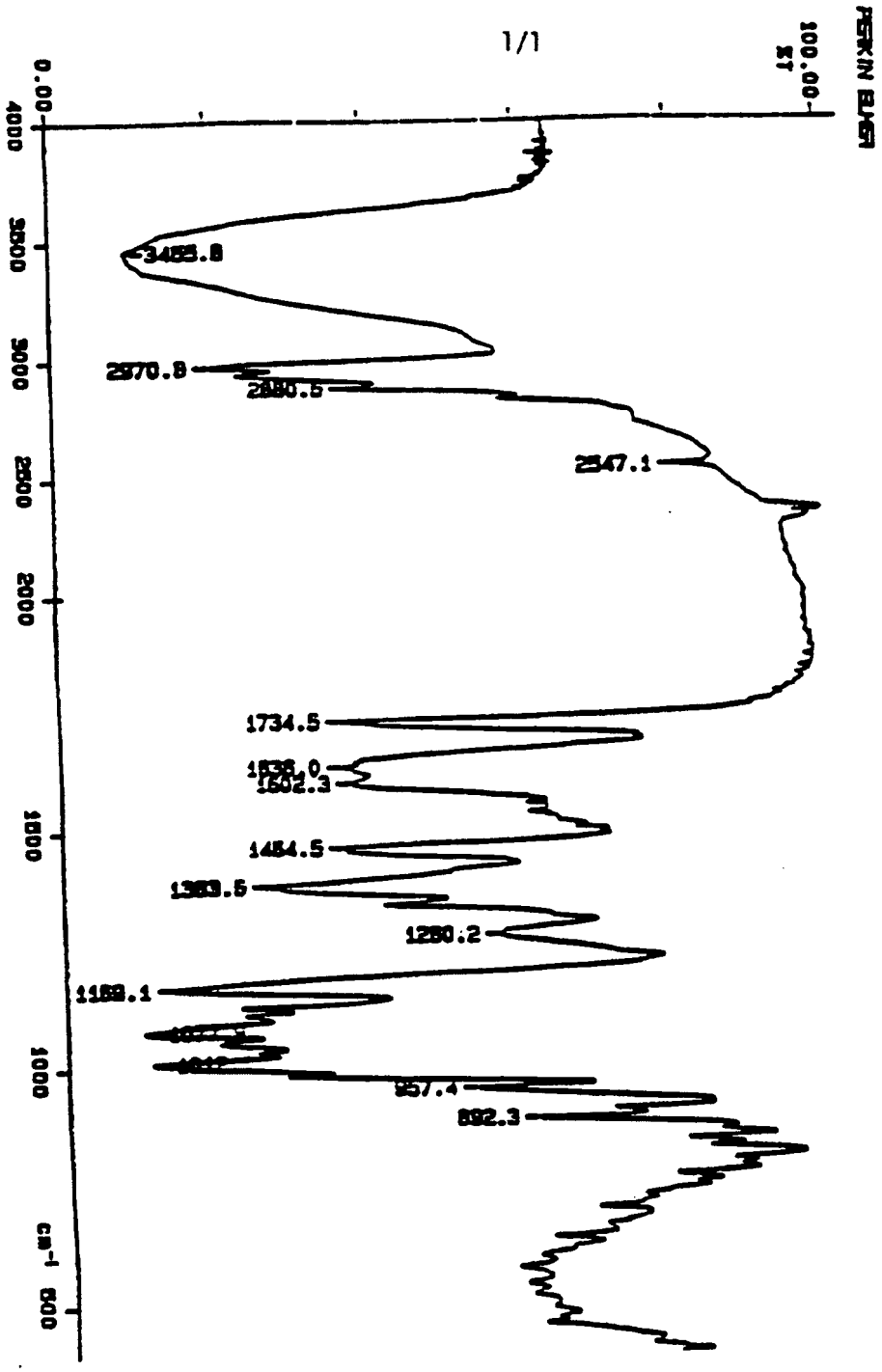
8. A process as claimed in claim 7 wherein the reaction is carried out in an organic solvent.

9. A process as claimed in claim 7 or 8 wherein the reaction is carried out in an aqueous medium.

10. A process as claimed in any of claims 7 to 9 wherein the reaction is carried out at a temperature of from 20°C to 40°C.

11. A process substantially as hereinbefore described with reference to the Examples.

12. A compound whenever prepared by a process as claimed in any of claims 7 to 11.



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Acetylcysteine Roxithromycin Salt

Fig. 1

INTERNATIONAL SEARCH REPORT

Internatic Application No
PCT/IE 95/00065

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H17/08 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 057 489 (REFARMED RECH PHARM MED) 11 August 1982 cited in the application see the whole document ---	1-12
Y	INT. J. CLIN. PHARMACOL. THER. TOXICOL., vol. 26, 1988, pages 444-7, XP002002013 M. DE BERNARDI ET AL.: "Human pharmacokinetics of erythryomycin propionate-N-acetylcysteinate" see the whole document ---	1-12

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
8 May 1996	30 -05- 1996

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	ANNAL. PHARMACOTHER., vol. 26, 1992, pages 46-55, XP002002015 N. BAHAL AND M.C. NAHATA: "The new macrolide antibiotics: azithromycin, clarithromycin, dirithromycin and roxithromycin" see the whole document	1-12
Y	J. CLIN. MICROBIOL., vol. 5, 1977, pages 58-61, XP002002481 M.F. PARRY AND H. NEU: "Effect of N-Acetylcysteine on Antibiotic Activity and Bacterial Growth In Vitro" see the whole document	1-12
A	FR,A,2 534 588 (ROUSSEL-UCLAF) 20 April 1984 see the whole document	1-12

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Information on patent family members

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		EP-A,B	0060573	22-09-82
		EP-A,B	0174395	19-03-86
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FR-A-2534588	20-04-84	NONE		